Homoflavonoids from *Ophioglossum petiolatum*

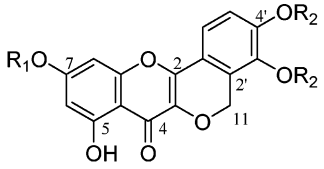
Yun-Lian Lin,* Chien-Chang Shen, Yeh-Jeng Huang, and Ying-Ying Chang

National Research Institute of Chinese Medicine, Taipei 112, Taiwan

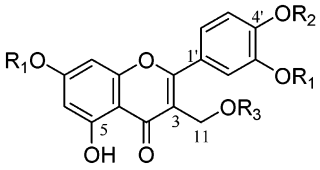
Received August 20, 2004

Six homoflavonoids, ophioglonin (**1**), ophioglonin 7-*O*-*β*-D-glucopyranoside (**2**), ophioglonol (**3**), ophioglonol prenyl ether (**4**), ophioglonol 4′-*O*-*β*-D-glucopyranoside (**5**), and isoophioglonin 7-*O*-*β*-D-glucopyranoside (**6**), together with five known compounds, quercetin, luteolin, kaempferol, 3,5,7,3′,4′-pentahydroxy-8-prenylflavone, and quercetin 3-*O*-methyl ether, were isolated from *Ophioglossum petiolatum*. Their structures were elucidated by analysis of spectroscopic data and chemical evidence. Compounds **1** and quercetin 3-*O*-methyl ether showed slight anti-HBV surface antigen activity at 25 *μ*M.

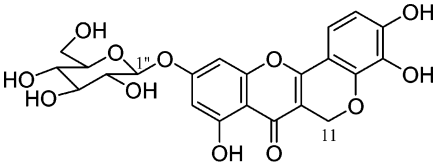
Ophioglossum petiolatum Hook (Ophioglossaceae) is a small terrestrial plant that is distributed worldwide.[1] It has been used as a detoxifying agent[2] and claimed to be effective against hepatitis in the herbal medicine of Taiwan. Although *O. petiolatum* has been used in traditional Chinese medicine, there has been no previous chemical studies on this plant. In this study, six new homoflavonoids (**1**−**6**) and five known flavones were isolated from the whole plant. Herein, we describe the isolation and structure elucidation of the six homoflavonoids.



1 $R_1 = R_2 = H$
2 R_1 = β-D-glucopyranosyl, $R_2 = H$
7 $R_1 = R_2 = Me$



3 $R_1 = R_2 = R_3 = H$
4 $R_1 = R_2 = H$, R_3 =
5 $R_1 = R_3 = H$, R_2 = β-D-glucopyranosyl
8 $R_1 = R_2 = Me$, $R_3 = H$



6

Results and Discussion

The ethanolic extract of the dried whole plant of *O. petiolatum* was partitioned between ethyl acetate and *n*-butanol successively. The EtOAc fraction and *n*-BuOH fractions were separated by silica gel and Sephadex LH-20 column chromatography to yield six homoflavonoids, ophioglonin (**1**), ophioglonin 7-*O*-*β*-D-glucopyranoside (**2**), ophioglonol (**3**), ophioglonol prenyl ether (**4**), ophioglonol 4′-*O*-*β*-D-glucopyranoside (**5**), and isoophioglonin 7-*O*-*β*-D-glucopyranoside (**6**), together with five known compounds, quercetin,[3] luteolin,[4] kaempferol,[3] 3,5,7,3′,4′-pentahydroxy-8-prenylflavone,[5] and quercetin 3-*O*-methyl ether.[6]

Compound **1** was obtained as greenish yellow needles. The molecular formula was established as $C_{16}H_{10}O_7$ from HREIMS at *m/z* 314.0475 and ^{13}C NMR with 12 indices of hydrogen deficiency (IHD). The IR spectrum showed hydroxyl (3433, 1202, and 1031 cm^{-1}), conjugated carbonyl (1662 cm^{-1}), and aromatic (1624, 1600, and 1508 cm^{-1}) absorptions. The UV spectrum was similar to those of flavonols with two absorption bands (384 and 260 nm).[7] Analysis of the ^1H NMR spectrum revealed the presence of one methylene [δ_H 5.16 (s); δ_C 63.0 (t)], two *meta*-coupled [δ_H 6.15 and 6.41 (1H each, d, J = 2.0 Hz)] and two *ortho*-coupled aromatic protons [δ_H 6.88 and 7.17 (1H each, d, J = 8.5 Hz)], three phenolic hydroxy groups [δ_H 9.17, 10.36, and 10.80 (1H each, br s)], and a hydrogen-bonded hydroxy group (δ_H 12.74). The ^{13}C NMR, DEPT, and HMQC analyses showed an oxymethylene carbon, seven aromatic carbons with three fully substituted sp^2 carbons, seven oxygenated aromatic carbons, and a carbonyl carbon. The HMBC spectrum of **1** showed cross-peaks from H-11 to C-3, C-1′, C-2′, and C-3′; from H-5′ to C-1′, C-3′, C-4′, and C-6′; and from H-6′ to C-2, C-1′, C-2′, C-4′, and C-5′. They confirmed the assignments for H-11, C-2/3, and the B-ring. Partial methylation of **1** with diazomethane in dried acetone gave a tri-*O*-methyl ether (**7**) as an amorphous powder, and its ^1H NMR spectrum showed methoxy signals at δ 3.88 (6H) and 3.94 (3H) and a hydrogen-bonded hydroxy group at δ 12.62. The ^1H NMR spectrum was similar to that of a photoproduct from quercetin pentamethyl ether[8] except for the hydrogen-bonded hydroxy group in place of a methoxy group. The NOE effect between 5′-H and 4′-OMe (δ 3.94), between 11-H and 3′-OMe (δ 3.88), and between 6-H and 8-H and 7-OMe (δ 3.88) confirmed the location of the hydroxy groups in **1**. From the NOE and HMBC correlations, ophioglonin can be assigned the structure **1**. This homoflavonoid has not previously been reported from natural sources.

The molecular formula of compound **2** was suggested as $C_{22}H_{20}O_{12}$ on the basis of HRFABMS. The ^1H and ^{13}C NMR spectra (Table 1) showed a coupling pattern similar to that of compound **1** with the exception of signals due to a *β*-D-glucopyranosyl moiety [δ_H 5.00 (d, J = 7.5 Hz, anomeric-H); δ_C 100.0 (C-1″), 72.9 (C-2″), 76.3 (C-3″), 69.4 (C-4″), 77.1 (C-5″), 60.5 (C-6″)] instead of a hydroxy group in **1**.

* To whom correspondence should be addressed. Tel: + 2-2820-1999, ext. 6531. Fax: + 2-2825-0743. E-mail: yllin@cma23.nricm.edu.tw.

10.1021/np0401819 CCC: $30.25
Published on Web 03/05/2005

Table 1. ^{1}H and ^{13}C NMR Data of **1**−**6** (500 and 125 MHz in DMSO-d_6)

	1		**2**		**3**		**4**		**5**		**6**	
position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		149.7 s		149.7 s		164.4 s		165.4 s		163.5 s		161.1 s
3		132.9 s		133.0 s		117.0 s		114.2 s		117.3 s		113.9 s
4		174.1 s		174.1 s		181.4 s		181.1 s		180.9 s		175.8 s
5		161.5 s		161.2 s		161.6 s		161.5 s		161.4 s		161.2 s
6	6.15 d (2.0)	98.6d	6.40 d (2.0)	99.0 d	6.19 d (2.0)	98.7 d	6.16 d (2.0)	99.0 d	6.19 d (2.0)	99.6 s	6.33 d (2.0)	98.7 s
7		163.7 s		162.4 s		164.6 s		165.5 s		161.5 s		161.6 s
8	6.41d (2.0)	93.8 d	6.70 d (2.0)	94.4 d	6.35 d (2.0)	93.6 d	6.32 d (2.0)	93.8 d	6.36 d (2.0)	94.1 d	6.66 d (2.0)	94.2 d
9		155.9 s		155.3 s		157.2 s		157.2 s		157.4 s		154.9 s
10		104.5 s		106.0 s		103.1 s		102.6 s		102.1 s		105.8 s
11	5.16 s	63.0 t	5.20 s	63.0 t	4.30 s	53.6 t	4.20 s	61.8 t	4.28 d (12.0) 4.31 d (12.0)	53.5 t	5.05 s	63.3 t
1′		119.8 s		119.6 s		122.7 s		122.3 s		126.1 s		116.8 s
2′		115.5 s		115.1 s	7.34 d (2.0)	116.1 d	7.25 d (2.0)	115.9 d	7.40 d (2.0)	116.4 d		114.5 d
3′		140.7 s		140.6 s		145.2 s		145.4 s		146.7 s		129.8 s
4′		148.9 s		149.4 s		148.8 s		149.2 s		147.9 s		143.0 s
5′	6.88 d (8.5)	114.8 d	6.90 d (8.5)	114.7 d	6.88 d (8.5)	115.4 d	6.87 d (8.0)	115.3 d	7.26 d (8.5)	115.6 d	6.34 d (8.5)	113.9 d
6′	7.17 d (8.5)	114.2 d	7.19 d (8.5)	114.1 d	7.30 dd (8.5, 2.0)	121.2 d	7.22 dd (8.0,2.0)	121.2 d	7.38 dd (8.5)	120.5 d	7.08 d (8.5)	116.3 d
1″			5.00 d (7.5)	100.0 d			4.02 d (7.0)	66.4 t	4.86 d (7.0)	101.6 d	5.00 d (7.5)	100.0 d
2″				72.9 d			5.37 t (7.0)	121.4 d		73.2 d		73.1 d
3″				76.3 s				135.9 s		75.9 d		76.4 d
4″				69.4 d			1.71 s	25.5 q		69.8 d		69.6 d
5″				77.1 d			1.66 s	17.8 q		77.3 d		77.1 d
6″				60.5 t						60.7 t		60.6 t
OH	9.17 br s 10.36 br s 10.80 br s 12.74 br s		9.08 br s 10.28 br s 12.72 br s		13.06 br s 5.28 t		12.98 br s		9.07 br s 10.95 br s 12.99 br s		13.00 br s	

The glycosidation at C-7 was determined from the lower field shift of H-6 (δ 6.40) and H-8 (δ 6.70) and the higher field shift (1.3 ppm) of C-7; NOESY correlations between H-1″ and H-6 and H-8; and HMBC correlations between H-1″ and C-7 and between H-6 and H-8 and C-7. Structure **2** could thus be assigned as ophioglonin 7-*O*-β-D-glucopyranoside.

Ophioglonol (**3**) was obtained as a yellow amorphous powder with a molecular formula of $C_{16}H_{12}O_7$ from HREIMS (*m/z* 316.0560) and ^{13}C NMR data with 11 IHD. The IR spectrum suggested the presence of hydroxyls (3448 and 3231, 1180, 1115, 1086 cm^{-1}), a conjugated carbonyl (1662 cm^{-1}), and an aromatic system (1611, 1561, and 1508 cm^{-1}). The UV maxima observed at 333 and 251 nm suggested a flavonoid skeleton.[7] The ^{1}H NMR spectrum revealed the presence of two *meta*-coupled aromatic protons [δ_H 6.19 and 6.35 (1H each, d, J = 2.0 Hz))] assigned to H-6 and H-8, an aromatic ABX system [δ_H 6.88 (d, J = 8.5), 7.30 (dd, J = 8.5, 2.0 Hz), 7.34 (d, J = 2.0 Hz)] indicating the presence of a 1,3,4-trisubstituted phenyl group, methylene protons bearing an oxygen function [δ_H 4.30 (s)], and a hydrogen-bonded hydroxyl at δ 13.06. The ^{13}C (Table 1) and DEPT NMR spectra showed 16 signals including one oxymethylene carbon, five sp^2 methine carbons, nine sp^2 quarternary carbons with six oxygenated carbons, and a carbonyl. C−H long-range HMBC correlations between H-11 and C-2, C-3, and C-4 suggested the location of a hydroxymethyl moiety at C-3. Further confirmation by a NOESY experiment showed NOE correlations between H-11 and H-2′ and H-6′. Partial methylation of **3** by diazomethane afforded the tri-*O*-methyl ether (**8**) as colorless needles. The ^{1}H NMR spectrum of **8** showed methoxy signals at 3.95, 3.96, and 3.97 and a hydrogen-bonded hydroxy group at δ 12.96. In the NOE experiments, irradiation of the signal at δ 6.39 (H-6) resulted in the enhancement of the methoxy signal at δ 3.97, and irradiation of the signal at δ 6.97 (d, J = 8.5 Hz, H-5′) and 7.26 (d, J = 2.0 Hz, H-2′) caused an enhancement of the methoxy group at δ 3.96 (4′-OMe) and 3.95 (3′-OMe), respectively. From the above spectroscopic data and further HMBC correlations of **3** from the hydrogen-bonded hydroxyl to C-5, C-6, and C-10; from H-8 to C-6, C-7, C-9, and C-10; and from H-6′ to C-2, C-1′, C-2′, C-4′, and C-5′, the structure of ophioglonol was established as **3**.

The UV and ^{1}H and ^{13}C NMR spectra of **4** and **5** were similar to those of **3**. However, the spectrum of compound **4** showed a 3-methylbut-2-enyloxymethyl [δ_H 4.20 (2H, s), 4.02 (2H, d, J = 7.0 Hz), 5.37 (1H, t, J = 7.0 Hz), 1.66 and 1.71 (3H each, s); δ_C 61.8, 66.4, 121.4, 135.9, 17.8, and 25.5] in place of the hydroxymethyl moiety at C-3 in **3**. Long-range correlation of H-1″ and C-11 confirmed the location of the 3-methylbut-2-enyloxymethyl moiety in **4**. Compound **5** appeared to have a β-D-glucopyranosyl moiety [δ_H 4.86 (d, J = 7.0 Hz); δ_C 101.6, 77.3, 75.9, 73.2, 69.8, 60.7] instead of a hydroxy group as in **3**. Long-range correlation of H-1″ and C-4′ and an NOE correlation of the anomeric proton [δ_H 4.86 (d, J = 7.0 Hz)] and H-5′ [δ_H 7.26 (d, J = 8.5 Hz)] revealed the position of glycosidation at C-4′ as in compound **5**.

Compound **6** was isolated as a yellow amorphous powder, and its molecular formula was assigned as $C_{22}H_{20}O_{11}$ on the basis of its HRFABMS, with 13 IHD. The IR spectrum showed the presence of hydroxyl (3420, 1204, and 1075 cm^{-1}), aromatic system (1597 cm^{-1}), and conjugated car-

bonyl (1655 cm^{-1}) absorption bands. The ^1H and ^{13}C NMR spectra were similar to those of **2**, except that the *ortho*-coupled aromatic protons were shifted upfield [δ_H 6.34 and 7.08 (1H each, d, J = 8.5 Hz)] and the carbonyl moved to δ_C 175.8. The HMBC spectrum of **6** revealed long-range correlations between H-11 and C-2, C-3, C-4, and C-2′ and between H-1″ and C-7, confirming **6** as the proposed structure.

All of the isolated compounds were tested for antihepatitis B virus (HBV) activity using the MS-G2 hepatoma cell line. Compound **1** and quercetin 3-*O*-methyl ether showed slight anti-HBV surface antigen secretion at 25 μM.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrophotometer. UV spectra were measured on a Hitachi U-3310 spectrophotometer. NMR spectra were run on a Varian Unity INOVA-500 spectrometer. Mass spectra (EIMS, HREIMS, and HRFABMS) were taken on a JEOL JMS-100 and JEOL SX-102A instrument, respectively.

Plant Material. The whole plants of *O. petiolatum* were collected from Taipei County, Taiwan, in May 2003. The plant was identified by comparison with the voucher specimens deposited earlier at the Herbarium of the Department of Botany, National Taiwan Unversity, Taipei, Taiwan (no. TAI000271)

Extraction and Isolation. The dried whole plants of *O. petiolatum* (1.2 kg) were extracted three times with 95% EtOH (each 3 L) under reflux. The combined EtOH extract was evaporated under reduced pressure. The concentrate (115 g) was suspended in H_2O and partitioned with EtOAc (× 3) and *n*-BuOH (× 3), successively. The EtOAc fraction (41 g) was subjected to silica gel column chromatography using a C_6H_{14}−$CHCl_3$−MeOH gradient. Fractions of 5−15% MeOH−$CHCl_3$ were then separated over a Sephadex LH-20 column and eluted with MeOH. Each fraction was further purified repeatedly by passage over a Sephadex LH-20 column (MeOH) to afford **1** (136 mg), **3** (45 mg), **4** (28 mg), quercetin (46 mg), luteolin (32 mg), kaempferol (26 mg), 3,5,7,3′,4′-pentahydroxy-8-prenylflavone (38 mg), and quercetin 3-*O*-methyl ether (68 mg). The *n*-BuOH fraction (45 g) was directly subjected to Diaion HP-20 column chromatography using a gradient of 60% MeOH−H_2O to MeOH gradient. Fractions of 80% MeOH/H_2O−MeOH were then separated on a Sephadex LH-20 column and eluted with MeOH. Each fraction was further purified by passage over a Sephadex LH-20 column (MeOH) to afford **2** (23 mg), **5** (26 mg), and **6** (21 mg).

Ophioglonin (1): greenish yellow needles from ethanol, mp 275−277 °C (dec); IR (KBr) ν_{max} 3433, 1662, 1624, 1600, 1508, 1290, 1228, 1202, 1031, 968, 810, 700, 637 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 384 (3.96), 275 sh (3.55), 260 (4.01) nm; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; HMBC correlations, H-6/C-5, C-7, C-8, C-10; H-8/C-6, C-7, C-9, C-10; H-11/C-3, C-1′, C-2′, C-3′; H-5′/ C-1′, C-2′, C-3′, C-4′; H-6′/C-2, C-1′, C-2′, C-4′, C-5′; O*H*-5/C-5, C-6, C-10; NOESY correlations, O*H*-7/H-6, H-8, O*H*-4′/H-5′; EIMS *m/z* 314 $[M]^+$ (100), 302 (15), 286 (20); HREIMS *m/z* 314.0475 (calcd for $C_{16}H_{10}O_7$, 314.0426).

Ophioglonin 7-*O*-β-D-glucopyranoside (2): yellow amorphous powder; IR (KBr) ν_{max} 3424, 1655, 1624, 1582, 1503, 1293, 1204, 1173, 1075, 810, 700 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 382 (3.94), 278 sh (3.52), 260 (4.02) nm; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; HMBC correlations, H-6/C-5, C-7, C-8, C-10; H-8/C-6, C-7, C-9, C-10; H-11/C-3, C-1′, C-2′, C-3′; H-5′/C-1′, C-2′, C-3′, C-4′; H-6′/C-2, C-1′, C-2′, C-4′, C-5′; H-1″/C-7; O*H*-5/C-5, C-6, C-10; NOESY correlations, H-1″/H-6, H-8, O*H*-4′/H-5′; FABMS *m/z* 477 $[M + H]^+$; HRFABMS *m/z* 477.1011 (calcd for $C_{22}H_{20}O_{12}$, 477.1032).

Ophioglonol (3): pale yellow amorphous powder; IR (KBr) ν_{max} 3448, 3231, 1662, 1611, 1561, 1508, 1180, 1115, 1086, 964, 810 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 333 (3.83), 262 (3.97), 251 (4.00) nm; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz): see Table 1; HMBC correlations, H-6/C-5, C-7, C-8, C-10; H-8/C-6, C-7, C-9, C-10; H-11/C-2, C-3, C-4; H-2′/C-2, C-1′, C-3′, C-4′, C-6′; H-5′/C-1′, C-3′, C-4′, C-6′; H-6′/C-2, C-1′, C-2′, C-3′, C-4′, C-5′; NOESY correlations, H-11/H-2′, H-6′; EIMS *m/z* 316 $[M]^+$ (25), 298 (85), 286 (100); HREIMS *m/z* 316.0560 (calcd for $C_{16}H_{12}O_7$, 316.0543).

Ophioglonol prenyl ether (4): pale yellow amorphous powder; IR (KBr) ν_{max} 3280, 1656, 1609, 1504, 1293, 1172, 1125, 1078, 973, 820 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 331 (3.82), 261 (4.01), 256 (4.02) nm; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; HMBC correlations, H-6/C-5, C-7, C-8, C-10; H-8/C-6, C-7, C-9, C-10; H-11/C-2, C-3, C-4; H-2′/C-2, C-1′, C-3′, C-4′, C-6′; H-5′/C-1′, C-3′, C-4′, C-6′; H-6′/ C-2, C-1′, C-2′, C-3′, C-4′, C-5′; H-1″/ C-11, C-2″, C-3″; H-2″/C-1″, C-3″, C-4″, C-5″; NOESY correlations, H-11/H-2′, H-6′; EIMS *m/z* 384 $[M]^+$ (8), 298 (35), 192 (76); HREIMS *m/z* 384.1172 (calcd for $C_{21}H_{20}O_7$, 384.1209).

Ophioglonol 4′-*O*-β-D-glucopyranoside (5): pale yellow amorphous powder; IR (KBr) ν_{max} 3424, 1655, 1624, 1582, 1503, 1293, 1204, 1173, 1075, 810, 700 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 322 (3.80), 263 (4.03), 248 (4.00) nm; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; HMBC correlations, H-6/C-5, C-7, C-8, C-10; H-8/C-6, C-7, C-9, C-10; H-11/C-2, C-3, C-4; H-5′/C-1′, C-3′, C-4′, C-6′; H-6′/C-2, C-1′, C-2′, C-4′, C-5′; H-1″/C-4′; NOESY correlations, H-1″/H-5′; H-11/H-2′, H-6′; FABMS *m/z* 479 $[M + H]^+$; HRFABMS *m/z* 479.1199 (calcd for $C_{22}H_{23}O_{12}$, 479.1189).

Isophioglonin 7-*O*-β-D-glucopyranoside (6): pale yellow amorphous powder; IR (KBr) ν_{max} 3420, 1655, 1597, 1291, 1204, 1173, 1075, 805, 705 cm^{-1}; UV (MeOH) λ_{max} (log ϵ), 380 (3.79), 260 (4.01) nm; ^1H NMR (DMSO-d_6, 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; HMBC correlations, H-6/C-5, C-7, C-8, C-10; H-8/C-6, C-7, C-9, C-10; H-11/C-2, C-3, C-4, C-2′; H-5′/C-1′, C-3′, C-4′, C-6′; H-6′/C-2, C-1′, C-2′, C-4′, C-5′; H-1″/C-7; NOESY correlations, H-1″/H-6, H-8; FABMS *m/z* 477 $[M + H]^+$; HRFABMS *m/z* 477.1014 (calcd for $C_{22}H_{20}O_{12}$, 477.1032).

Partial Methylation of 1 and 3. A mixture of compound **1** (3 mg), acetone (1 mL), and excess diazomethane (5 mL) was placed on an ice bath and stirred for 4 h. The solvent was evaporated in vacuo. The residue was purified by preparative TLC (2% MeOH−$CHCl_3$) to give **7**. Compound **7**: ^1H NMR ($CDCl_3$, 500 MHz) δ 3.88 (6H, s, 4′-, 7-OMe), 3.94 (3H, s, 3′-OMe), 5.32 (2H, s, H-11), 6.35 and 6.46 (1H each, d, J = 2.0, Hz, H-6, H-8), 6.95 and 7.50 (1H each, d, J = 8.5 Hz, H-5′, H-6′), 12.62 (1H, br s, OH). Methylation of **3** as above yielded **8**. Compound **8**: ^1H NMR ($CDCl_3$, 500 MHz) δ 3.95 (3H, s, 3′-OMe), 3.96 (3H, s, 4′-OMe), 3.97 (3H, s, 7-OMe), 4.62 (2H, s, H-11), 6.39 and 6.50 (1H each, d, J = 2.0, Hz, H-6, H-8), 6.97 (1H, d, J = 8.5 Hz, H-5′), 7.26 (1H, d, J = 2.0 Hz, H-2′), 7.30 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 12.96 (1H, br s, OH).

Antihepatitis B Virus (HBV) Assay. Hepatitis B virus producing hepatoma cell line MS-G2 was used as a target cell, which was constructed by Sureau et al.[11] Cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 IU/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L L-glutamine, 1% nonessential amino acids, and 2.5 μg/mL fungizone. For the antiviral assay, cells were plated onto 24-well culture dishes at a density of 3 × 10^5 cells/mL/well. All tested crude extracts and pure compounds were dissolved in DMSO at concentrations of 200 μg/mL for crude extracts and 50 μM for pure compounds as stock solutions. The concentration of DMSO in the media was maintained at no more than 2.5 μL/mL by sequential dilution to ensure that it did not affect the growth of MS-G2 cells. The culture media were collected on day 3 for each antiviral assay. The antiviral activities were evaluated by determining the changes in HBsAg and HBeAg level in the presence or absence of test compounds as analyzed by an enzyme-linked immunosorbant assay (ELISA) (EverNew, Co., Taipei, Taiwan). The results were read at wavelength 492 nm with a DYNATECH MR7000 enzyme-linked immunosorbant assay reader (Guernsey, Channel Islands, UK). The percentage inhibition (%) was calculated

by comparison with the DMSO solvent control group. % of inhibition = [1 − OD(492 nm) of sample well/OD (492 nm) of DMSO well] × 100. The results of three repeats were expressed as the mean ± standard deviation of the mean (SDM). Inhibition between 25 and 35% was defined as slight inhibition, 35−50% as medium inhibition, 50−65% as strong inhibition, while that over 65% was defined as very strong inhibition. Cell damage was assessed by the AST (aspartate transaminase) assay kit (Fuji kit). AST values higher than 25 IU/L served as an indication of cell damage. [12]

Acknowledgment. This work was supported by the National Science Council of the Republic of China (NSC 93-2320-B-077-001).

References and Notes

(1) *Flora of Taiwan*, 2nd ed.; Editorial Committee of the Flora of Taiwan: Taipei, 1994; Vol I, p 71.
(2) Kao, M. T. *Popular Herbal Remedies of Taiwan*; Southern Materials Center, Inc.: Taipei, 1985; p 21.
(3) Lin, Y. L.; Wang, W. Y.; Kuo, Y. H.; Chen, C. F. *J. Chin. Chem. Soc.* **2000**, *47*, 247−251.
(4) Lin, Y. L.; Wang, W. Y. *Chin. Pharm. J.* **2002**, *54*, 187−192.
(5) Fukai, T.; Nomur, T. *Heterocycles* **1992**, *34*, 1213−1225.
(6) Stevens, J. F.; Wollenweber, E.; Ivancic, M.; Hsu, V. L.; Sundberg, S.; Deinzer, M. L. *Phytochemistry* **1999**, *51*, 771−780.
(7) Mary, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970; p 41.
(8) Waiss, A. C.; Lundin, R. E., Jr.; Lee, A.; Corse, J. *J. Am. Chem. Soc.* **1967**, *89*, 6213−6218.
(9) Brandt, E. V.; Ferreira, D.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 514−521.
(10) Malan, E.; Roux, D. G. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2696−2703.
(11) Sureau, C.; Romet-Lemonne, J. L.; Mullins, J. I.; Essex, M. *Cell* **1986**, *47*, 37−47.
(12) Huang, R. L.; Chen, C. C.; Huang, Y. L.; Hsieh, D. J.; Hu, C. P.; Chen, C. F.; Chang, C. M. *Hepatology* **1996**, *24*, 508−515.

NP0401819